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The NASA Twins Study:

The Effect of One Year in Space on Long-Chain Fatty Acid Desaturases and Elongases

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Short Title: Fatty Acid Desaturases & Elongases in Space

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1. Abstract

Background To date, there is no clear understanding of the effect of long-duration spaceflight on the major enzymes that govern the metabolism of omega-6 and omega-3 fatty acids. To address this gap in knowledge, we used data from the NASA Twins Study, which includes a multi-scale omic investigation of the changes that occurred during a year-long (340 days) human spaceflight. Embedded within the NASA Twins data are specific analytes associated with fatty acid metabolism.

Objectives To examine the long-chain fatty acid desaturases and elongases in a single human during one year in space.

Method One male twin was on board the International Space Station (ISS) for one year, while his monozygotic twin served as a genetically matched ground control. Longitudinal assessments included the genome, epigenome, transcriptome, proteome, metabolome, microbiome, and immunome during the mission, as well as six months before and after. The gene-specific fatty acid desaturase and elongase transcriptome data (FADS1, FADS2, ELOVL2 and ELOVL5) were extracted from untargeted RNA-seq measurements derived from white blood cell fractions.

Results

Most data from the elongases and desaturases exhibited relatively similar expression profiles (R^2 >0.6) over time for the CD8, CD19, and LD cell fractions, indicating overall conservation of function within and between the subjects. Both cell-type and temporal specificity was observed in some cases, and some differences were also apparent between the poly-adenylated fraction (polyA) of processed RNAs vs. the ribo-depleted (ribo-) fraction. The flight subject showed a stronger enrichment of the Fatty Acid Metabolic processes pathway across almost all cell types (columns, CD4, CD8, CPT, LD), most especially in the ribodepleted fraction of RNA, but also with the polyA+ fraction of RNA. GSEA enrichment measures across three related Fatty Acid Metabolism pathways showed a differential between the ground and flight subject.

Conclusions

There appears to be no persistent alteration of desaturase and elongase gene expression associated with one year in space. However, these data provide evidence that cellular lipid metabolism can be responsive and dynamic to spaceflight, even though it appears cell-type- and context-specific, most notably in terms of the fraction of RNA measured and the collection protocols. These results also provide new evidence of mid-flight spikes in expression of selected genes, which may indicate transient responses to specific insults during spaceflight.

2. Introduction

Future human exploration missions to Mars will include transit to and from the planet, with eventual exploration of the planet surface. These may be preceded or paralleled by lunar orbital or lunar colony missions of extended duration. The safe execution of such missions, including some lasting up to 500 days, will require a more detailed understanding of human physiology and aerospace medicine than has been available to date. Presently, only four humans have participated in spaceflight missions of one year or more. While our understanding of the biological consequences of 4- to 6-month missions on the ISS has increased substantially [1], there is almost no experience with exploration-class spaceflight. In addition, comprehensive molecular analytics, such as the multi-scale omics studies now common on Earth, have not previously been applied to humans on long-duration missions in space.

Longitudinal measures of the genome, epigenome, transcriptome, proteome, metabolome, and microbiome in space represents a considerable advancement in the ability to detect patterns of variance in molecular networks that are related to spaceflight. These new multi-scale omics platforms and metrics are also expected to aid in the identification of actionable biomarkers and targets from which to develop spaceflight countermeasures. Such knowledge would further allow space programs to develop truly personalized interventions, which would represent an evolution in spaceflight medicine [2].

There are presently no studies reporting on the biological response of long-chain, polyunsaturated fatty acid desaturase and elongase enzymes, and their associated metabolites, in astronauts who enter the space environment. Long-chain polyunsaturated fatty acids (PUFA) represent an important

class of molecules to study in spaceflight, because they shape the basic architecture of the lipid bilayer in all cell membranes. This includes shaping the membrane architecture of neurons, synapses, retina, and other highly electrically active elements of the brain [3]. It is noteworthy that the dry weight of the human brain is roughly 60% fat with an important dependence upon 20- and 22-carbon long-chain PUFA, especially docosahexaenoic acid (DHA) [4]. Given that the brain is increasingly being considered as one of the most vulnerable organs in the environment of space, understanding how the brain maintains and remodels the lipid fractions important to its architecture is likely among the more pressing items in spaceflight medicine [5].

Beyond structure, these long chain fatty acids act as crucial signaling molecules, regulators of inflammation, and pro-resolving mediators in the recovery process following injury [6]. Such mediators have a strong regulatory influence on systems relevant to space, such as the cardiovascular system [7]. Muscle catabolism and bone loss are two primary phenotypic changes that occur on exposure to microgravity in space. The role of omega-3 fatty acids, in particular eicosapentaenoic acid (EPA), has been extensively studied on Earth, with limited but substantive examination in space. Zwart *et al* have investigated the relationship between the intake of omega-3 fatty acids and bone-resorption markers in three models; 1) a cell culture system that models weightlessness, 2) *N*-telopeptide levels in healthy volunteers during 60 days of bed rest [1], and 3) the relationship between fish (and EPA) intake and *N*-telopeptide levels in astronauts on missions of 4 to 6 months on the International Space Station. The cell, bed rest, and flight data all support the hypothesis that EPA intake inhibited osteoclastogenesis. These models showed that the effect of EPA may be mediated, in part, via the NF-kB pathway [6]. Disuse atrophy of muscle is also associated with NF-kB activation, which similarly appears responsive to the modulating effect of omega-3 fatty acids [8].

Since selected fatty acids are essential and cannot be synthesized by the human body, understanding the dynamics of fatty acid metabolism in space is fundamental to forming proper exploration mission dietary regimens and clinical therapeutics [9]. Characterizing genetic variants of fatty acid metabolizing enzymes in an astronaut would further inform the application of individualized nutritional approaches in order to prevent specific fatty acid deficits, while optimizing for mission performance and health [10]. The fatty acids linoleic acid (18:2n6) and alpha-linolenic acid (18:3n3) are the two essential PUFA that, by definition, cannot be synthesized by humans and must be obtained from the diet. Their conversion to longer chain fatty acids (**Figure 1**) is governed by a series of steps that extend the fatty acid chain length by 2-carbon increments (elongation) and increase unsaturation by inserting double bonds (desaturation). Impairment in the elongation or desaturation enzymes can limit precursor fatty acid conversion, therein causing accumulation of precursor fatty acids or causing longer-chain fatty acids to become *conditionally essential*, thus requiring direct dietary supplementation or other interventions [11].

Fatty acid desaturases (FADS) are enzymes key to human metabolism that remove two hydrogen atoms from a fatty acid, creating a carbon-carbon double bond. Fatty acid desaturase 1 (FADS1; delta-5-desaturase) facilitates the direct conversion of omega-6 20:3n6 (DGLA) to 20:4n6 (AA). FADS1 also facilitates the direct conversion of omega-3 20:4n3 (ETA) to 20:5n3 (EPA). Fatty acid desaturase 2 (FADS2; delta-6-desaturase) facilitates the direct conversion of omega-6 18:2n6 (LA) to 18:3n6 (GLA). FADS2 also facilitates the direct conversion of omega-3 18:3n3 (ALA) to 18:4n3 (SDA). Additionally, FADS2 converts 24:4n-6 to 24:5n-6 and 24:5n-4 to 24:6n-3 prior to the final beta-oxidation step to produce 22:5n-6 and 22:6n-3, respectively. Collectively, these are the primary desaturation steps in converting long-chain polyunsaturated fatty acid precursors to their long-chain end products [12].

In addition, human elongases (ELOVL) are enzymes that extend the chain length of fatty acids by adding 2-carbon units during specific steps of fatty acid synthesis. There are two primary elongases in long-chain PUFA metabolism. These are elongase 2 (ELOVL2) and elongase 5 (ELOVL5). ELOVL5 facilitates the direct elongation of omega-6 18:3n6 (GLA) to 20:3n6 (DGLA). ELOVL5 also facilitates the direct elongation of omega-3 18:4n3 (SDA) to 20:4n3 (ETA). ELOVL2 facilitates the direct elongation of omega-6 20:4n6 (AA) to 22:4n6 (Adrenic). ELOVL2 also facilitates the direct elongation of omega-3 20:5n3 (EPA) to 22:5n3 (DPA). It is important to note that ELOVL5 can also participate in this conversion to omega-6 22:4n6 (Adrenic acid) and omega-3 22:5n3 (DPA). Similarly, ELOVL2 acts in a second step of the pathway to elongate 22:4n-6 to 24:4n-6 and 22:5n-3 to 24:5n-3. [13, 14]. The NASA Twins Study examined a wide range of molecular dynamics in space for a pair of identical twins (one on the ISS and one on Earth). Data were generated from specimens collected over a 25-month period, including before (preflight), during (inflight), and after (postflight) spaceflight. The resulting data represent NASA's first multi-scale omics profile of astronauts [15]. From this high-dimensional data set, we report for the first time on the targeted transcriptome of the genes that govern long-chain PUFA elongation and desaturation, and consider the implications for long-duration spaceflight.

3. Materials and Methods

Sample collection

A pair of monozygotic twins were studied for 25 months, during which one subject (Flight subject, TW) spent 340 days aboard the International Space Station (ISS) while his identical twin (Ground subject, HR) remained on Earth. Subjects were male and aged 50 at the beginning of the study. Both subjects had different amounts of spaceflight exposure prior to the study (Flight subject=180 days total, Ground subject=54 days total). Multiple blood samples were collected (N_{flight}=19, N_{ground}=12) from both subjects beginning approximately 6 months prior to the launch date, during the 340 days aboard the ISS and 6 months after return.

Transcriptome samples were either mixed by inversion and immediately frozen at -80°C (for both Earth and ISS collections), or were freshly collected, sorted, and processed. Fresh samples collected aboard the ISS were returned to Earth in the Soyuz capsule and kept at 4°C until processing (approximately 35 hours from collection to processing). Fresh samples were sorted into CD4, CD8, CD19 cell selection and the remaining Lymphocyte-depleted fraction (LD), while frozen samples were processed to isolate peripheral blood mononuclear cells (PBMC) from the CPT vacutainers. RNA was isolated from the samples and sequenced with RNA-seq using two separate library preparation methods (polyA selection and ribodepleted RNA). Blood sample collection, sorting, RNA extraction, quality control, library preparation and sequencing were performed as previously described [15].

All of the freshly collected samples aboard the ISS from the Flight subject were returned on the Soyuz capsule. Therefore, samples derived from ISS habitation were confounded by ambient return to

Earth. To correct for the effects of ambient return on gene expression, blood samples were collected from an unrelated individual, which were then exposed to conditions that simulated the fresh and ambient return collections. The fresh and ambient samples from the control individual were processed and sequenced with the same protocol used for the study subjects. Data generated from the control individual was used to model and correct the effects of ambient return, using ComBat [16, 17] or multivariate analyses in differential expression, as previously described [15].

Data Processing

Adapter removal was performed using cutadapt and Trim Galore! (v0.4.1) [18, 19]. Reads were aligned with STAR transcriptome aligner (v2.5.1b), using two-pass alignment to hg19 genome and Gencode v19 gene reference [20, 21]. Reads quantification was performed, using subread featureCounts and kallisto on ENSEMBL transcripts [22, 23, 24]. Transcript expression levels were summarized into their respective genes.

Differential gene expression analysis was performed using DESeq2 on gene counts [25]. Comparisons were done either within the Flight subject only, e.g. comparing samples collected preflight to inflight, or in a model that included samples from the Ground subject to model the normal baseline variance during the study duration. Ambient and fresh samples from the control individual were also included as a term in the multivariate model for any comparison that included ambient return samples. Each cell type was treated separately. Two different library preparations for polyA+ and rRNA depletion were combined together with an added covariate in the multivariate model. All differential gene expression analyses were performed, as previously described in 13. Gene Set Enrichment Analysis (GSEA) was performed using fgsea by ranking the genes on the resulting Wald statistic from DESeq2 and calculating enrichment on the Molecular Signatures Database (MSigDB) entries [26, 27, 28].

For normalized expression gene counts were normalized using trimmed mean of M (TMM) [29]. ComBat was applied to gene expression values including the fresh and ambient samples from the control subject to correct for the potential effect of ambient return on gene expression. Resulting normalized and corrected gene expression values were used for visualization and Gene Set Variation Analysis (GSVA) [30]. GSVA was performed for each cell type and library preparation method separately using the corrected gene expression values for MSigDB signatures. See Supplemental Methods for extensive detail on protocols used in the study.

4. Results

We examined the gene expression profiles of four key elongases and desaturases, specifically FADS1, FADS2, ELOVL2, and ELOVL5, as well as their overall biological pathways, represented from Gene Set Enrichment Analysis (GSEA) and Gene Set Variation Analysis (GSVA). These are represented by linear gene plots from before, during, and after the mission for the flight subject (TW), and compared to the ground control (HR) box plots summarizing the variance of the flight intervals and enrichment heat maps for the pathways.

Across these data, we observed several trends. First, most data from the elongases and desaturases exhibited relatively similar expression profiles (R^2 >0.6) over time for the CD8, CD19, and LD cell fractions, indicating overall conservation of function within and between the subjects. Yet, both cell-type and temporal specificity was observed in some cases, and some differences were also apparent between the poly-adenylated fraction (polyA) of processed RNAs vs. the ribo-depleted (ribo-) fraction, which represent more nascent transcripts. For example, the bulk-blood collection of peripheral blood mononuclear cells (PBMCs) from the CPT tubes showed a mid-mission, flight-specific increase in FADS1 (Figure 2, 3), in both RNA fractions. Yet, other genes showed more “spikes” in their expression, indicating a transient response associated with spaceflight or sensitivity to other

biological influence. This includes FADS2 (Figure 4,5), most notably in the polyA fraction of RNA, an increase in ELOVL2 during flight (Figure 6,7) in the polyA fraction, and a decrease in ELOVL5 during flight (Figure 8,9) in the polyA fraction.

To discern if related genes in these biochemical processes were also responsive to spaceflight, we examined the GSEA and GSVA enrichments for fatty acid metabolic pathways. The flight subject showed a stronger enrichment across almost all cell types (columns, CD4, CD8, CPT, LD) for the Fatty Acid Metabolic processes pathway, most especially in the ribodepleted fraction of RNA, but also with the polyA+ fraction of RNA (Figure 10). Also, the enrichment was nearly always above that of the ground control. Also, we used the GSEA enrichment measures across the related Fatty Acid Metabolism pathways, including Gene Ontology sets for "fatty acid derivative biosynthetic processes," "phosphate-containing compound metabolic processes," and "regulation of cell differentiation." All three of these GO sets showed a differential between the ground and flight subject, with a notable shift in the third flight sample (Figure 11). These represent both key process genes and related networks of genes that are dynamic in spaceflight.

5. Discussion/Conclusion

The NASA Twins Study represents the first integrated, multi-scale omics, molecular, physiological, and cognitive portrait of an astronaut engaged in a year-long space mission, which revealed myriad phenotypic responses of the human body. Our results are the first to demonstrate transient gene expression profiles of fatty acid desaturases and elongases in space, through examination of the transcriptome of FADS1, FADS2, ELOVL2 and ELOVL5 derived from untargeted RNA-seq analysis. Based on the transcriptome analysis presented herein, there appears to be no persistent alteration of these fatty acid metabolizing enzymes associated with one year in space, even though the second half of the mission (months 6-12) showed more differentially expressed genes (15). However, we observed that the enrichment of fatty acid metabolic pathway processes were already "primed" before spaceflight, and variable expression patterns across cell types. These patterns were isolated from CD4, CD8, and CD19 cell types, the remaining Lymphocyte-depleted fraction (LD), and peripheral blood mononuclear cells (PBMC).

These data provide evidence that cellular lipid metabolism can be responsive and dynamic during spaceflight, and that it can also be quite cell-type- and context-specific, most notably in terms of the fraction of RNA measured and the collection protocols. This is consistent with other studies that have examined expression patterns by cell type [31,32,33]. These results also provide new evidence of mid-flight spikes in expression of these key genes, analogous to those observed for DNA repair and immune activation, which may indicate transient responses to specific radiation, vascular, or physiologic insults during spaceflight. Moreover, the enrichment and differential pathways utilized as part of fatty acid metabolism also suggest potential new mechanisms of adaptation by the body, during long-duration spaceflight.

Several limitations of this study warrant consideration. First, the small number of subjects and large number of variables (analytes) renders such high-dimensional data prone to overfitting. One method to address this limitation is through serial measures of the baseline, in-flight, and post-flight conditions, which was the approach used in this study. Second, the cohort of males is limiting on the generalizability of these results to females. Nonetheless, the matched cohort favors homogeneity, which is valuable in studies involving high-dimensional data. Third, the data reported herein does not contain SNP, indel, CNV, and structural genomic elements for reasons of confidentiality. In particular, SNP variants of FADS1, FADS2, ELOVL2, and ELOVL5 would have been additionally informative, as

their impact on the conversion of precursor fatty acids to downstream metabolites has been characterized [15]. Thus, this analysis provides some insight into changes in molecular functional elements in the space environment, but it does not include an examination of structural genomic elements that might alter human risk in the space environment.

Fourth, we were unable to describe the precise relationship between fatty acid desaturases, elongases, and their fatty acid metabolites in space. In the original *Science* paper describing the first results of the Twins Study, it was reported that the *omega-6 20:4 fatty acid* (described as arachidonic acid; AA) increased, while lysophospholipids containing the omega-3 20:5 fatty acid (eicosapentaenoic acid; EPA) decreased in space [34]. While this represents a potentially important finding, our subsequent analysis revealed that AA was not individually isolated, as 20:4 consists of more than one isomer. More specifically, the 20:4 (eicosatetraenoic acid: ETA) measurement that was reported in the original methods did not differentiate between the omega-6 and omega-3 isomers of ETA (20:4), which includes the omega-6 ETA (*all-cis*-5,8,11,14-eicosatetraenoic acid) and the omega-3 ETA (*all-cis*-8,11,14,17-eicosatetraenoic acid). Thus, while it is likely that AA may have increased (as AA commonly predominates over ETA), the percent composition of AA in relation to ETA could not be established, and thus warrants higher-resolution methods in future studies.

Likewise, in reporting the 22:5 as docosapentaenoic acid (DPA), the analysis did not differentiate between the two isomers of DPA, which includes the omega-6 DPA (*all-cis*-4,7,10,13,16-docosapentaenoic acid) and the omega-3 DPA (*all-cis*-7,10,13,16,19-docosapentaenoic acid). Similarly, in reporting the 18:3 as linolenic acid, the analysis did not differentiate between the two isomers of linolenic acid, which includes the omega-6 γ-linolenic acid (*all-cis*-6,9,12-octadecatrienoic acid) and the omega-3 α-linolenic acid (*all-cis*-9,12,15-octadecatrienoic acid). While a general estimate of omega-6 trajectory in relation to omega-3 fatty acids was reported in the original *Science* paper, we were unable to complete a specific analysis of individual fatty acid metabolites in relation to elongase and desaturase enzymes, because of insufficient data surrounding the identity of individual fatty acid isomers.

Our ongoing work, using the extant data set, is examining the elongase and desaturase proteins, single nucleotide polymorphisms in elongase and desaturase genes, and epigenomic changes in genes that govern fatty acid metabolism, derived from the multi-scale omics analysis of one year in space. This work also explores fatty acid molecular dynamics in relation to various clinical phenotypes. Included is an examination of phenotypes that were observed to have changed during the one-year mission, such as cognitive, mitochondrial, immunological, cardiovascular, ocular, and other phenotypes. Potential correlations between fatty acid trajectories and cognitive associations will be of particular interest, since 1) cognitive speed decreased for all tests (except for the visual object learning test) after one year in space, 2) accuracy decreased for 80% of tested domains postflight, and 3) fatty acids have previously been associated with changes in cognition [15].

In the future, new methods for direct sequencing and molecular assays that can be performed directly on the ISS can help reduce some of the confounding variables [35-37], as well as provide longer sequence reads and improved resolution of isoform changes [38]. The use of red blood cell fatty acid analysis is expected to provide a more stable measure of fatty acid status that is more reflective of tissue stores and shows less day-to-day variation associated with fatty acid intake. If serum or whole blood is used, strict controls related to dietary intake and fasting blood samples should be employed. Isolation of individual fatty acid isomers within each group (e.g. 18:3, n6/n3; 20:4, n6/n3; 22:5, n6/n3, etc.) using methods such as GC-FID or GC-MS, will add further precision. Incorporating the analysis of eicosanoids, docosanoids, and pro-resolving mediators (down-stream metabolites of long-chain PUFA) will further elucidate an important class of signaling and regulatory

derivatives of fatty acids. New dietary monitoring methods, tracking of dietary supplement intake, and pre-flight quantification of the ISS foods can further aid in the identification of the potential drivers of the variations in fatty acid molecular networks. Since double-bonded fatty acids are uniquely sensitive to oxidation, such as that initiated by ionizing radiation, improved ISS radiation monitoring would be of benefit.

The degree to which the present study of one year in low Earth orbit accurately reflects the biological dynamics that would emerge from a three-year mission to Mars or prolonged lunar habitation, is currently unknown. Therefore, the future support of exploration-class missions will benefit from additional multi-scale omics studies of longer duration and varying conditions.

6. Appendix Glossary of Terms

Omega-6

LA: linoleic acid (18:2n-6)

GLA: γ-linolenic acid (18:3n-6)

DGLA: dihomo-γ-linolenic acid (20:3n-6)

AA: arachidonic acid (eicosatetraenoic acid; 20:4n-6)

DTA: docosatetraenoic acid (22:4n-6)

DPAn-6: docosapentaenoic acid (22:5n-6)

TTA: tetracosatetraenoic acid (24:4n-6)

TPAn-6: tetracosapentaenoic acid (24:5n-6)

Omega-3

ALA: α-linolenic acid (18:3n-3)

SDA: stearidonic acid (18:4n-3)

ETA: eicosatetraenoic acid (20:4n-3)

EPA: eicosapentaenoic acid (20:5n-3)

DPAn-3: docosapentaenoic acid (22:5n-3)

DHA: docosahexaenoic acid (22:6n-3)

TPAn-3: tetracosapentaenoic acid (24:5n-3)

THA: tetracosahexaenoic acid (24:6n-3)

Enzymes

FADS1: fatty acid desaturase 1

FADS2: fatty acid desaturase 2

ELOVL2: Elongation of very long chain fatty acids protein 2

ELOVL5: Elongation of very long chain fatty acids protein 5

8. Statements

All papers must contain the following statements after the main body of the text and before the reference list:

8.1. Acknowledgement

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8.2. Statement of Ethics

The study participants have given their written informed consent. The study protocol has been approved by the research institute's (both NASA and WCM) committee on human research, specifically IRB# 1309014347.

8.3. Disclosure Statement

CEM is a cofounder and board member for Biotia, Inc., and Onegevity Health, Inc.

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8.5. Author Contributions

Michael Schmidt and Christopher Mason conceived the overall study objectives. Michael Schmidt and Christopher Mason led the manuscript writing, organization, and editing efforts. Christopher Mason and Cem Meydan processed the data, and Cem Meydan performed statistical analysis and multi-omics data integration. Michael Schmidt led molecular target selection and pathway elucidation. Michael Schmidt, Christopher Mason, Cem Meydan, Ebrahim Afshinnekoo, and Caleb Schmidt performed the data interpretation, translation to biological meaning, summary of results, discussion, conclusion, and future directions.

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Fig. 1. The Role of Elongases and Desaturases in the Synthesis of Long Chain Polyunsaturated Fatty Acids in Humans. ELOVL2 = Elongation of very long chain fatty acids protein 2; ELOVL5 = Elongation of very long chain fatty acids protein 5; FADS1 = Fatty acid desaturase 1; FADS2 = Fatty acid desaturase 2

Fig. 2. Gene Expression Profile for FADS1 Shown is the line plot expression data for fatty acid desaturase 1 (FADS1), including the PolyA+ and Ribodepleted specimens for various timepoints and cell types (CD4 CD8, CD19, lymphocyte-depleted fraction [LD], and CPT [peripheral blood mononuclear cells]), representing ground (green) and flight (blue).

Fig.3. Gene Expression Profile for FADS1 Shown is the box plot expression data for fatty acid desaturase 1 (FADS1), including the PolyA+ and Ribodepleted specimens for various timepoints and cell types (CD4 CD8, CD19, lymphocyte-depleted fraction [LD], and CPT [peripheral blood mononuclear cells]), representing ground (green) and flight (blue).

Fig. 4. Gene Expression Profile for FADS2 Shown is the line plot expression data for fatty acid desaturase 2 (FADS2), including the PolyA+ and Ribodepleted specimens for various timepoints and cell types (CD4 CD8, CD19, lymphocyte-depleted fraction [LD], and CPT [peripheral blood mononuclear cells]), representing ground (green) and flight (blue).

Fig. 5. Gene Expression Profile for FADS2 Shown is the box plot expression data for fatty acid desaturase 2 (FADS2), including the PolyA+ and Ribodepleted specimens for various timepoints and cell types (CD4 CD8, CD19, lymphocyte-depleted fraction [LD], and CPT [peripheral blood mononuclear cells]), representing ground (green) and flight (blue).

Fig. 6. Gene Expression Profile for ELOVL 2 Shown is the line plot expression data for fatty acid elongase ELOVL2, including the PolyA+ and Ribodepleted specimens for various timepoints and cell types (CD4 CD8, CD19, lymphocyte-depleted fraction [LD], and CPT [peripheral blood mononuclear cells]), representing ground (green) and flight (blue).

Fig. 7 Gene Expression Profile for ELOVL 2 Shown is the box plot expression data for fatty acid elongase ELOVL2, including the PolyA+ and Ribodepleted specimens for various timepoints and cell types (CD4 CD8, CD19, lymphocyte-depleted fraction [LD], and CPT [peripheral blood mononuclear cells]), representing ground (green) and flight (blue).

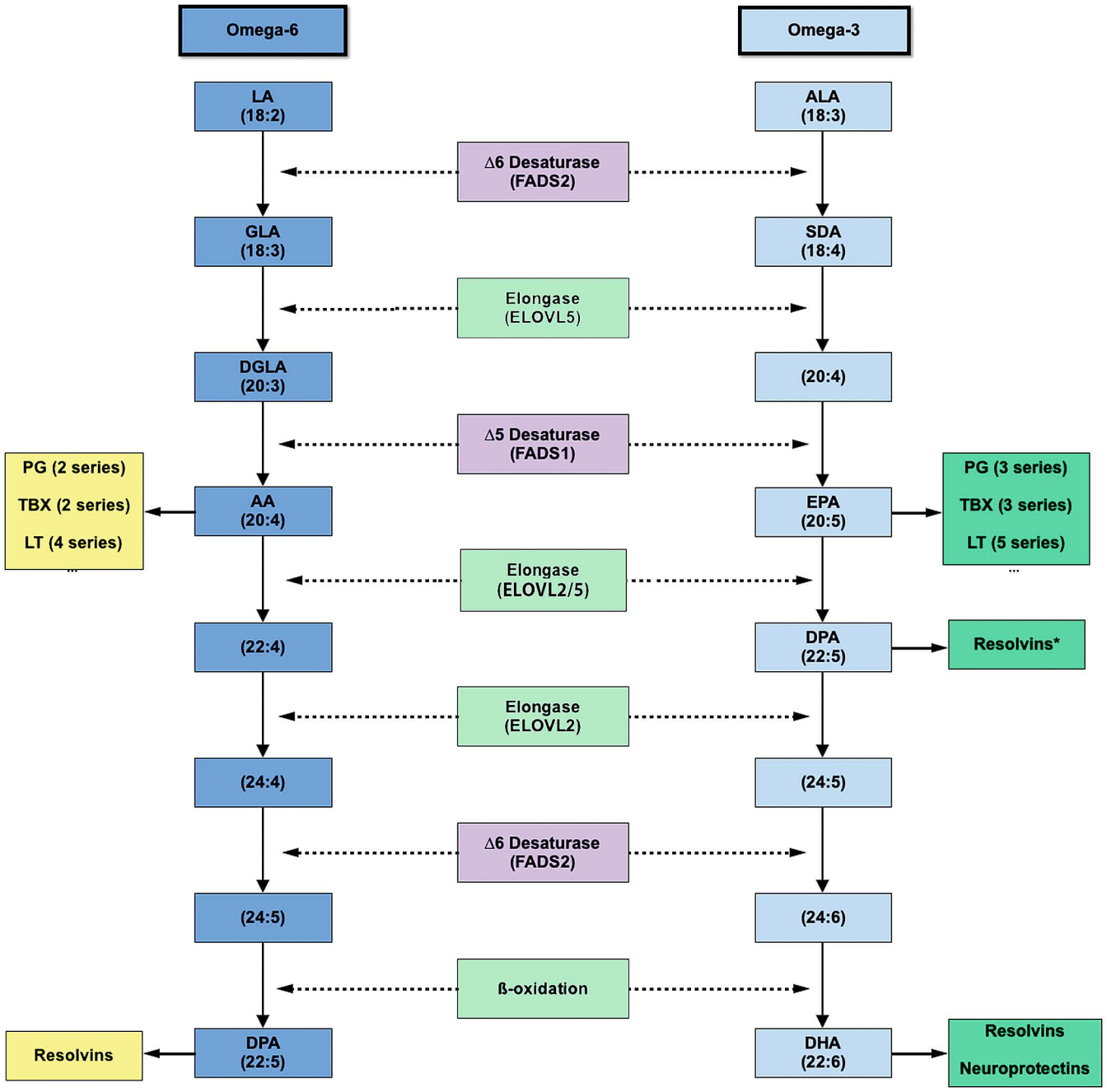
Fig. 8 Gene Expression Profile for ELOVL 5 Shown is the line plot expression data for fatty acid elongase ELOVL5, including the PolyA+ and Ribodepleted specimens for various timepoints and cell types (CD4 CD8, CD19, lymphocyte-depleted fraction [LD], and CPT [peripheral blood mononuclear cells]), representing ground (green) and flight (blue).

Fig 9. Gene Expression Profile for ELOVL 5 Shown is the box plot expression data for fatty acid elongase ELOVL5, including the PolyA+ and Ribodepleted specimens for various timepoints and cell types (CD4 CD8, CD19, lymphocyte-depleted fraction [LD], and CPT [peripheral blood mononuclear cells]), representing ground (green) and flight (blue).

Fig. 10: GSVA Pathway Enrichment for Fatty Acid Metabolic Processes. Data for the flight subject (blue) showed a stronger enrichment across almost all cell types (columns, CD4, CD8, CPT, LD) for the Fatty Acid Metabolic processes pathway, most especially in the ribodepleted fraction of RNA (bottom row), but also with the polyA+ fraction of RNA (top row).

Figure 11: Fatty Acid Metabolism Gene Ontology Differences. Plotted is the enrichment (positive=orange, negative=purple) for specific fatty acid pathways (rows) for various timepoints in

the study (top row labels), including pre-flight (dark purple), flight (blue), post-flight (green), and longitudinal ground control (brown). Ambient return samples (AR) are labelled in black.


Omega-6
Omega-3
LA (18:2)
ALA (18:3)
Δ6 Desaturase (FADS2)
GLA (18:3)
SDA (18:4)
Elongase (ELOVL5)
DGLA (20:3)
(20:4)
Δ5 Desaturase (FADS1)
PG (2 series)
TBX (2 series)
LT (4 series)
...
AA (20:4)
EPA (20:5)
PG (3 series)
TBX (3 series)
LT (5 series)
...
Elongase (ELOVL2/5)
(22:4)
DPA (22:5)
Resolvins*
Elongase (ELOVL2)
(24:4)
(24:5)
Δ6 Desaturase (FADS2)
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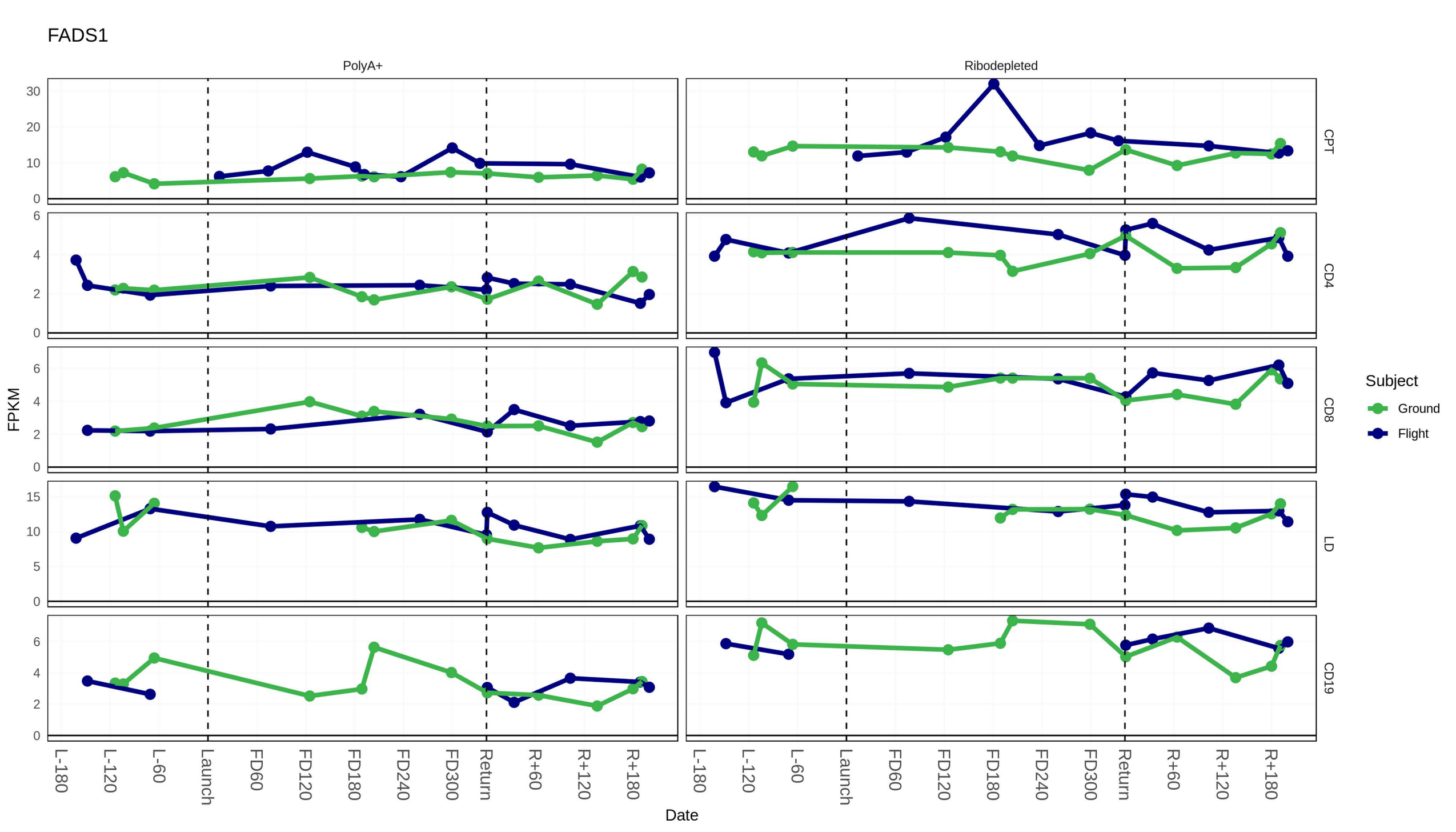
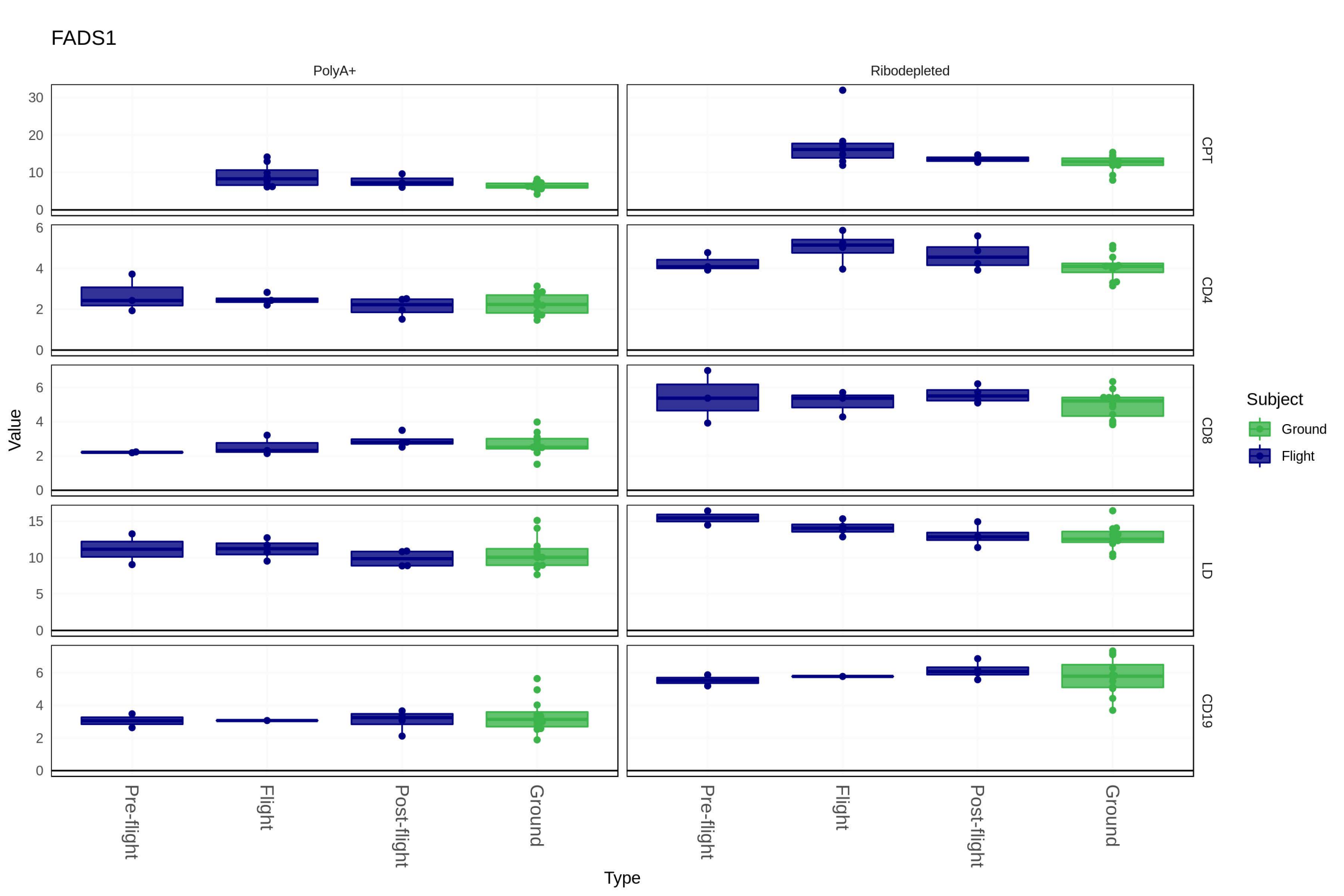
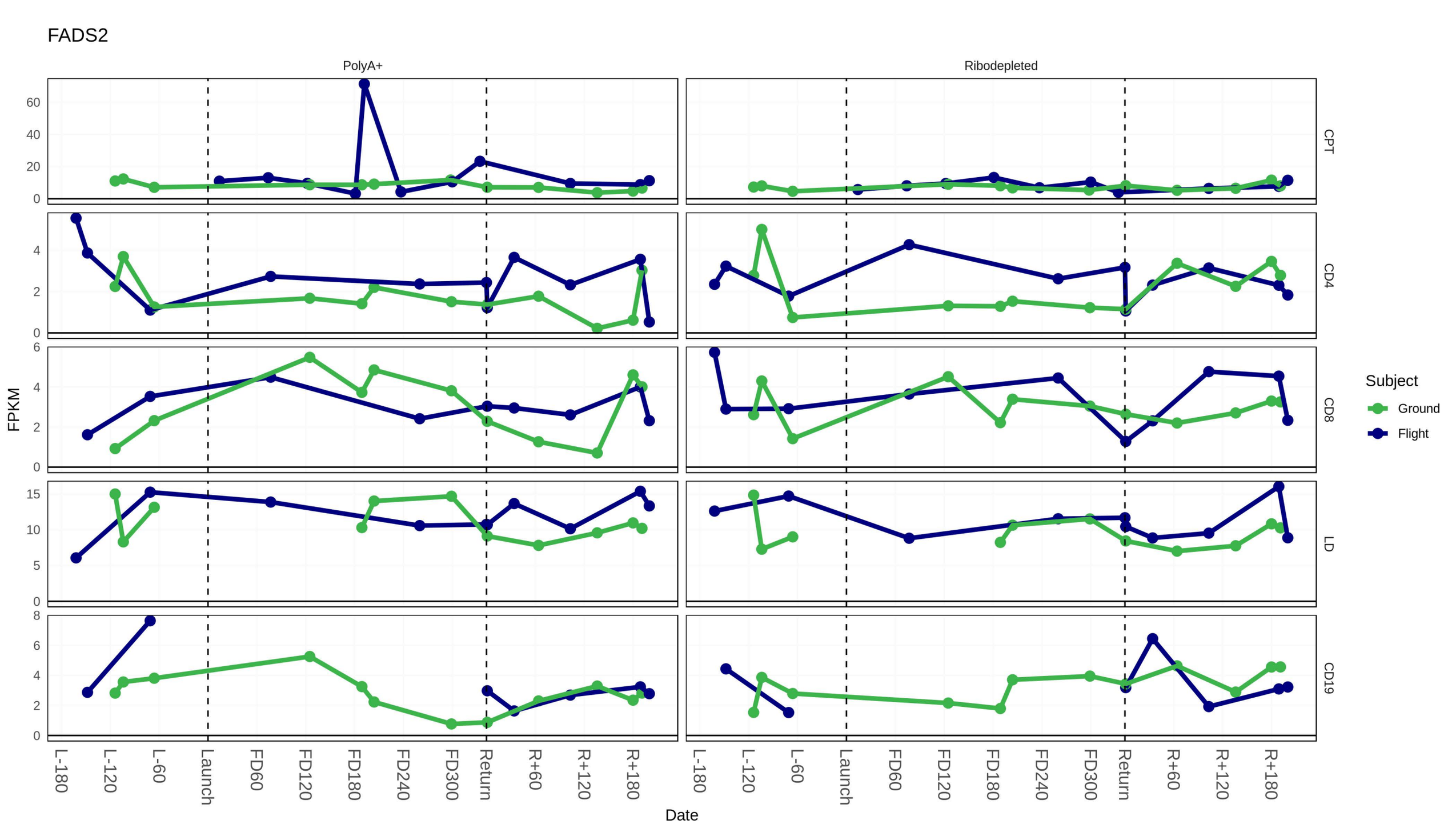
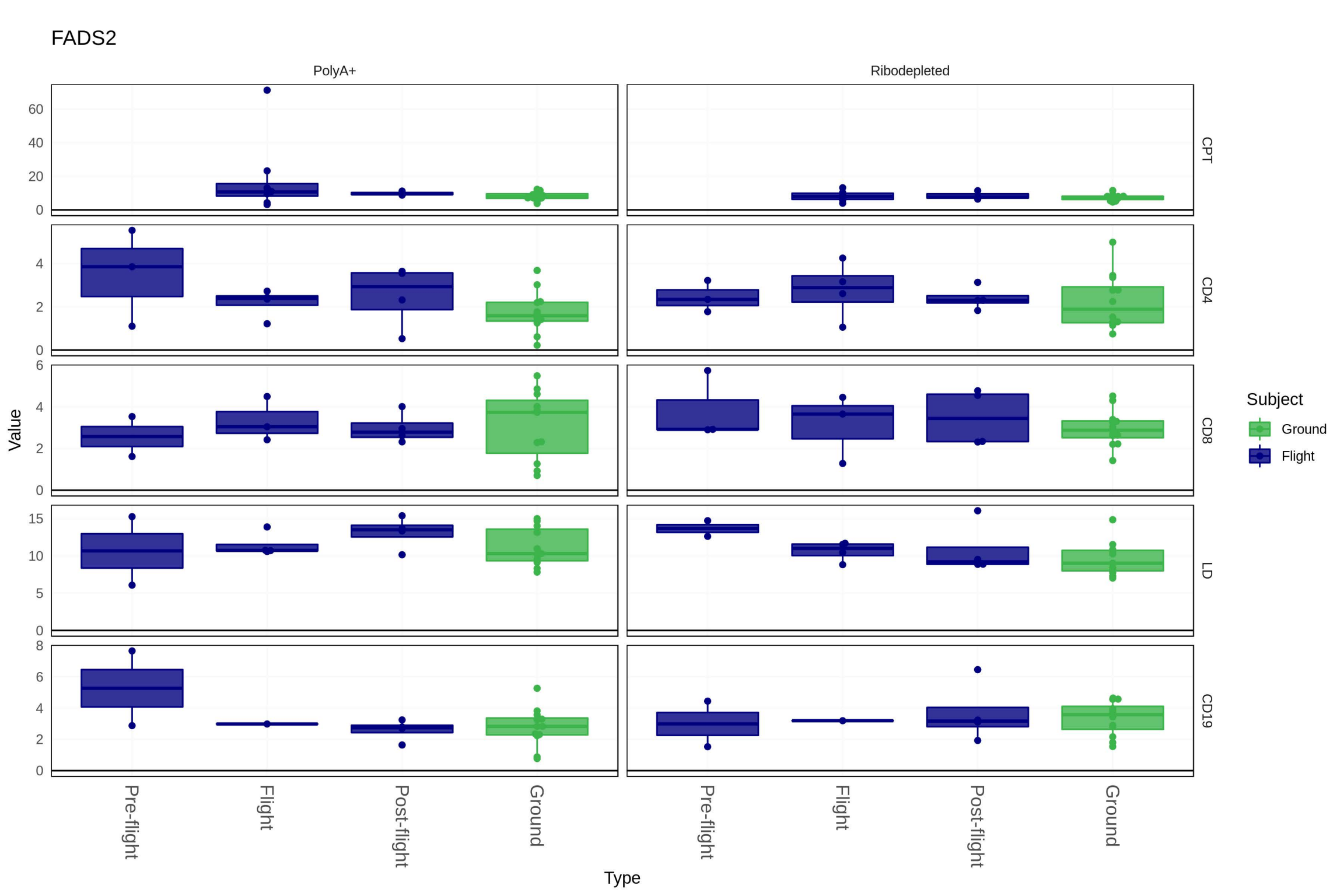
(24:6)
ß-oxidation
Resolvins
DPA (22:5)
DHA (22:6)
Resolvins
Neuroprotectins


FADS1
PolyA+
Ribodepleted
CPT
CD4
CD8
LD
CD19
FPKM
Date
Subject
Ground
Flight
L-180
L-120
L-60
Launch
FD60
FD120
FD180
FD240
FD300
Return
R+60
R+120
R+180


FADS1
PolyA+
Ribodepleted
CPT
CD4
CD8
LD
CD19
Value
Type
Pre-flight
Flight
Post-flight
Ground
Subject
Ground
Flight


FADS2
PolyA+
Ribodepleted
CPT
CD4
CD8
LD
CD19
FPKM
Date
Subject
Ground
Flight
L-180
L-120
L-60
Launch
FD60
FD120
FD180
FD240
FD300
Return
R+60
R+120
R+180


FADS2
PolyA+
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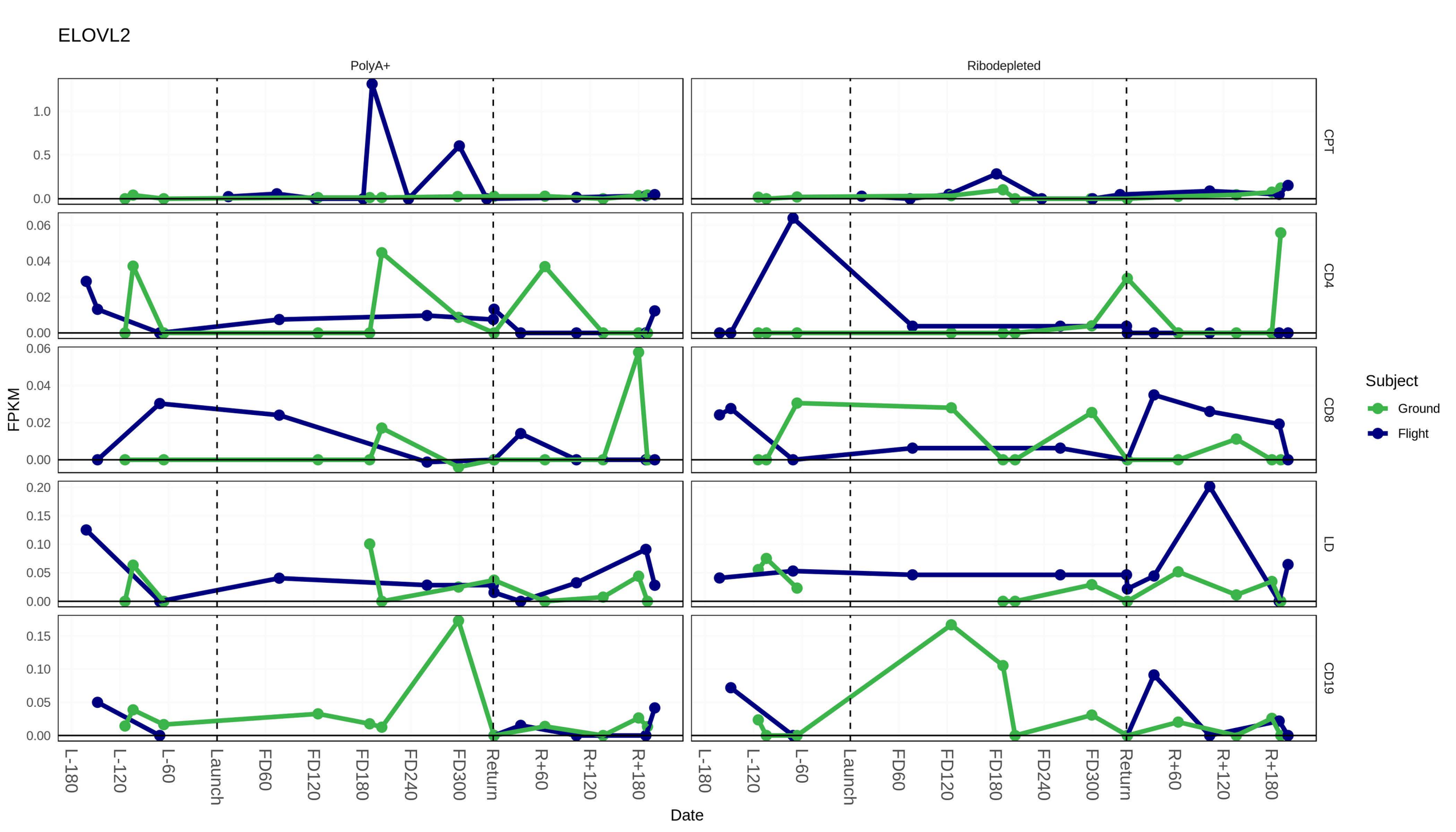
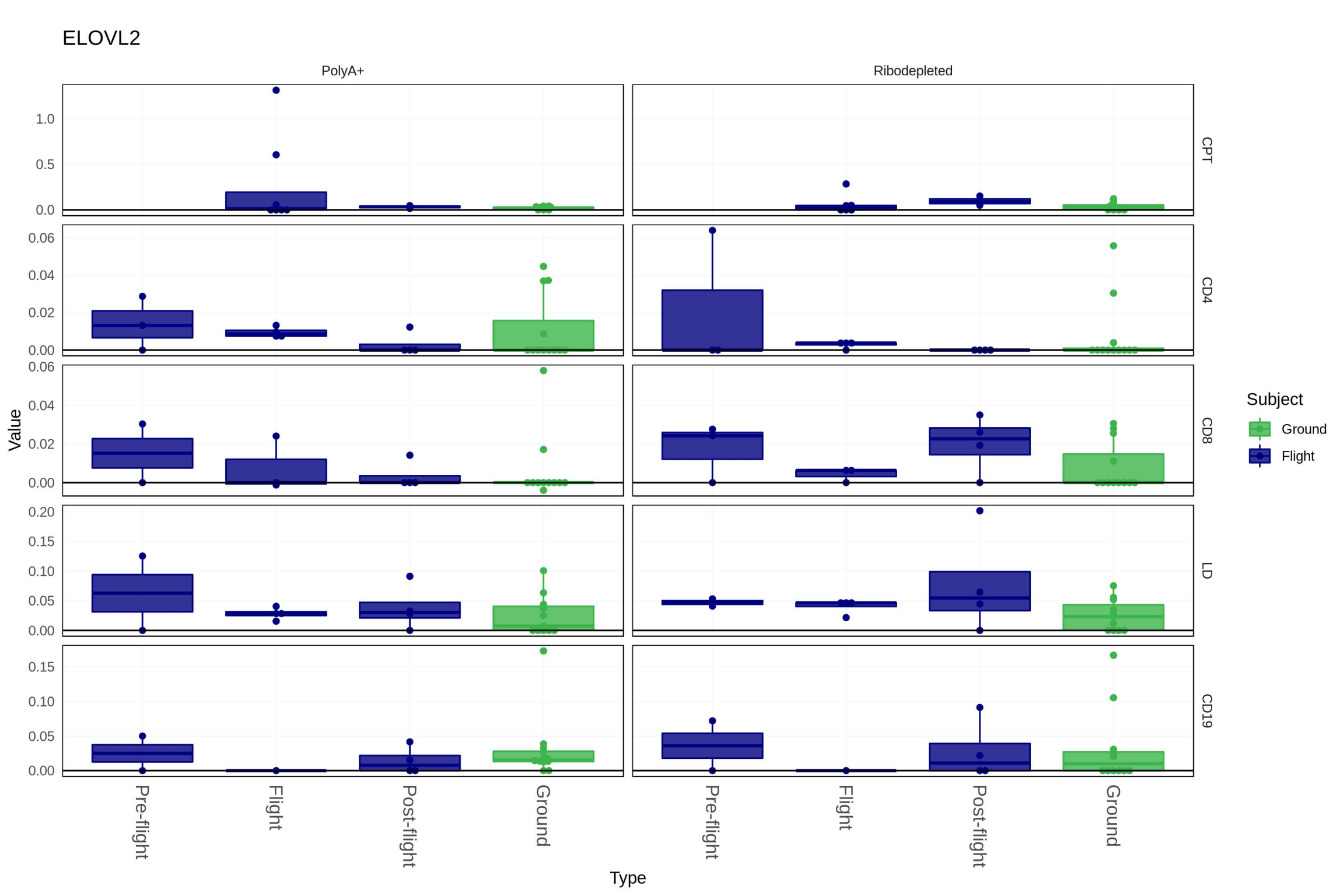
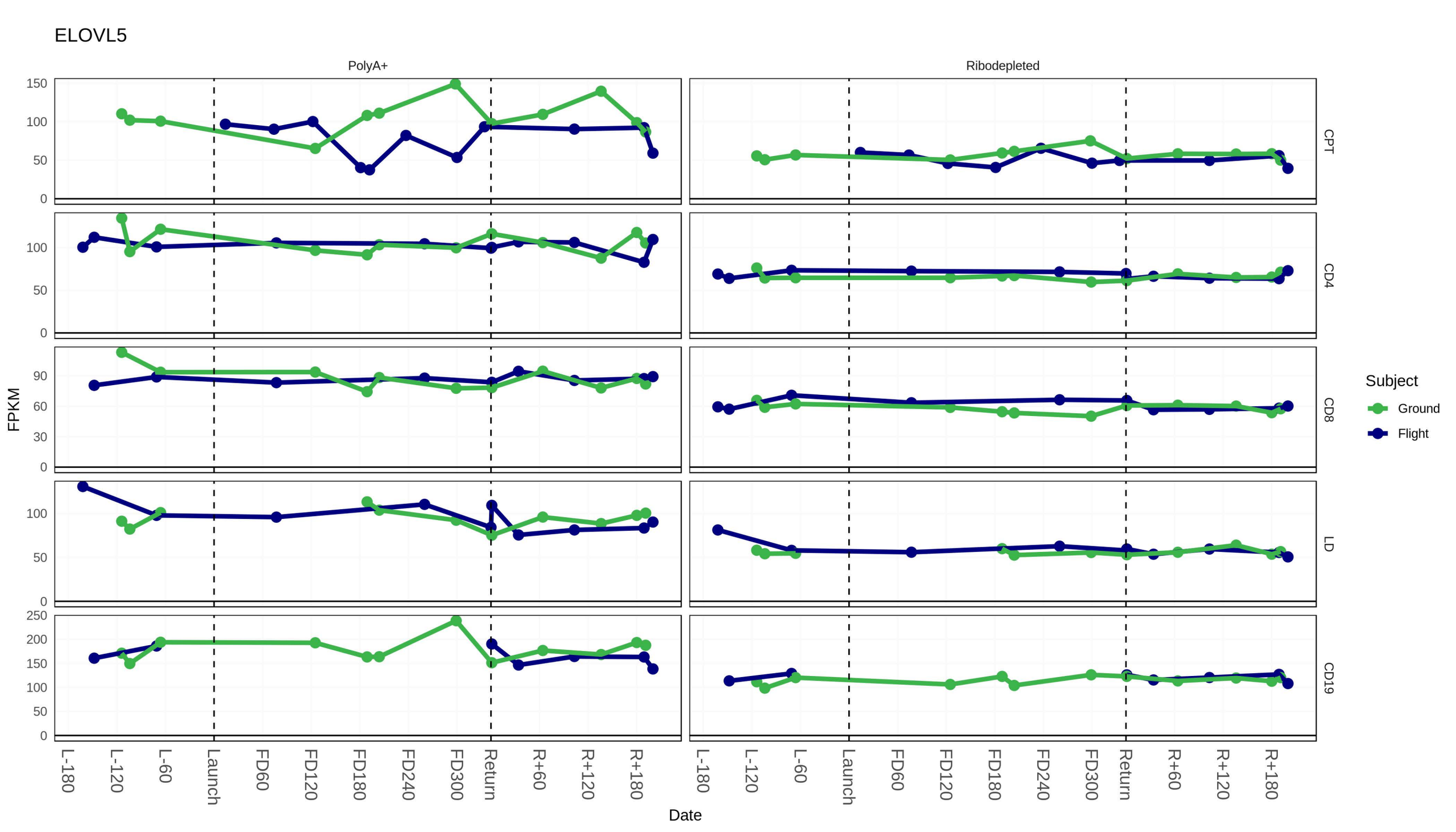
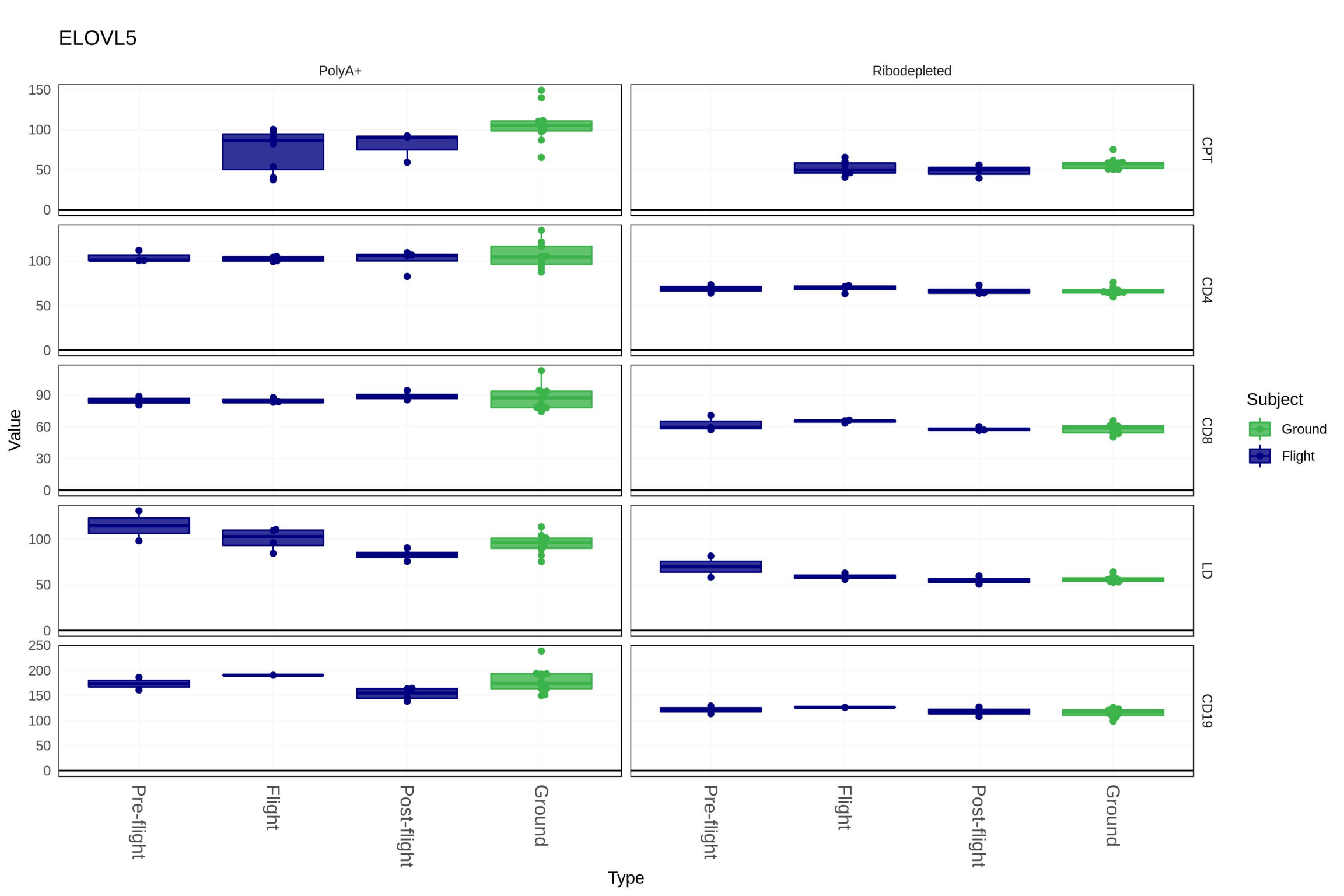
CPT
CD4
CD8
LD
CD19
Value
Type
Pre-flight
Flight
Post-flight
Ground
Subject
Ground
Flight

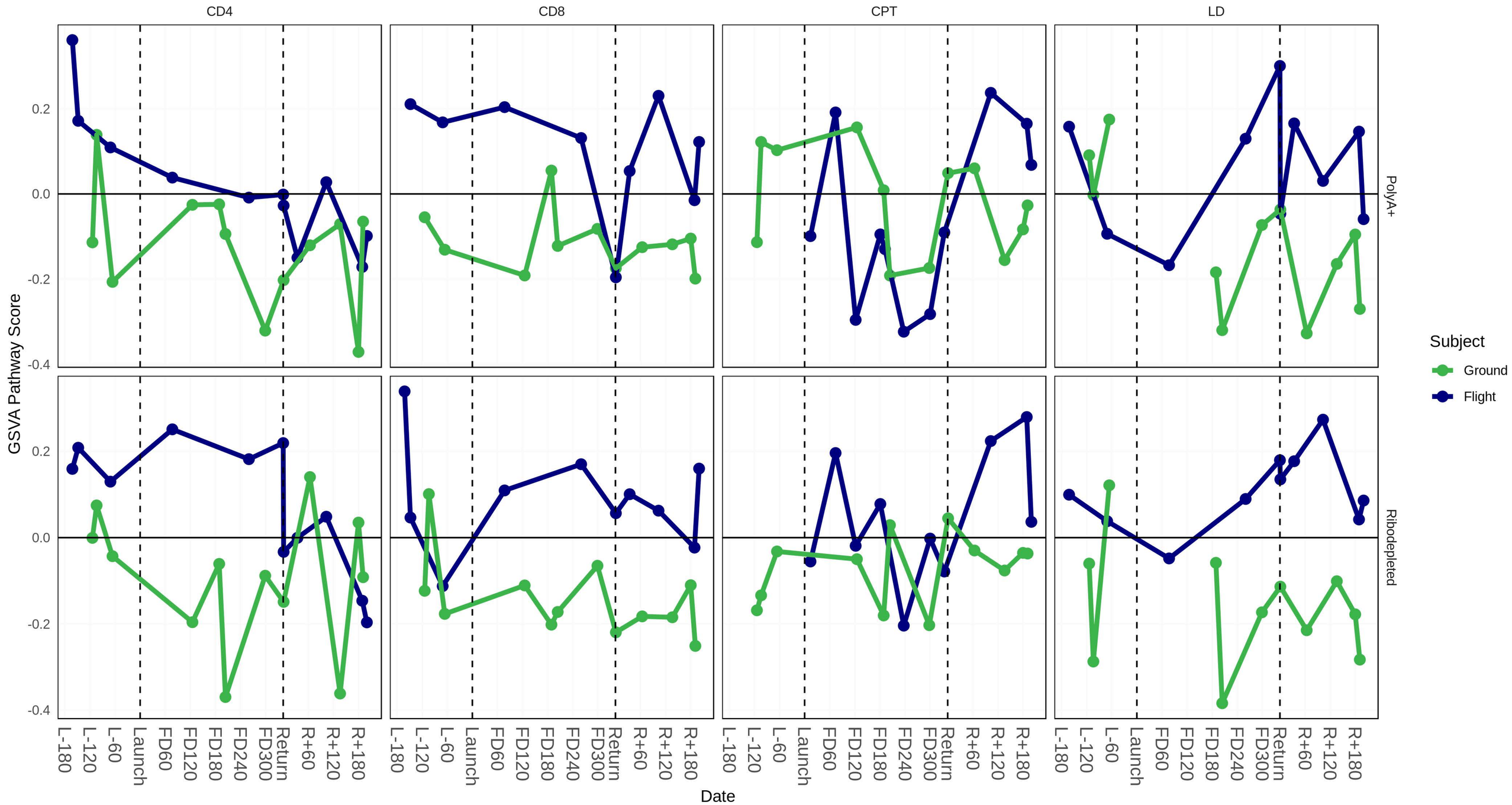

ELOVL2
PolyA+
Ribodepleted
CPT
CD4
CD8
LD
CD19
FPKM
Date
Subject
Ground
Flight
L-180
L-120
L-60
Launch
FD60
FD120
FD180
FD240
FD300
Return
R+60
R+120
R+180

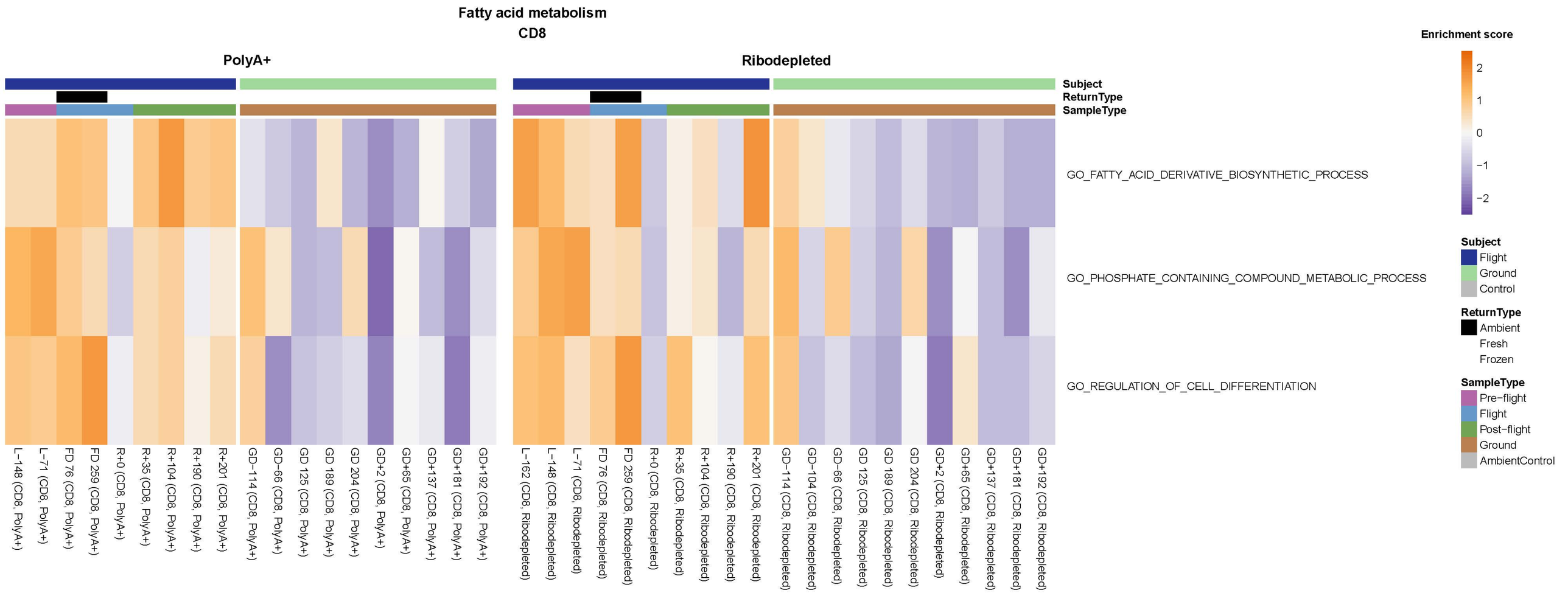

ELOVL2
PolyA+
Ribodepleted
CPT
CD4
CD8
LD
CD19
Value
Type
Pre-flight
Flight
Post-flight
Ground
Subject
Ground
Flight


ELOVL5
PolyA+
Ribodepleted
CPT
CD4
CD8
LD
CD19
FPKM
Date
Subject
Ground
Flight
L-180
L-120
L-60
Launch
FD60
FD120
FD180
FD240
FD300
Return
R+60
R+120
R+180


ELOVL5
PolyA+
Ribodepleted
CPT
CD4
CD8
LD
CD19
Value
Type
Pre-flight
Flight
Post-flight
Ground
Subject
Ground
Flight

GO_LONG_CHAIN_FATTY_ACID_METABOLIC_PROCESS




Fatty acid metabolism
CD8
PolyA+
Ribodepleted
Subject
ReturnType
SampleType
GO_FATTY_ACID_DERIVATIVE_BIOSYNTHETIC_PROCESS
GO_PHOSPHATE_CONTAINING_COMPOUND_METABOLIC_PROCESS
GO_REGULATION_OF_CELL_DIFFERENTIATION
L−148 (CD8, PolyA+)
L−71 (CD8, PolyA+)
FD 76 (CD8, PolyA+)
FD 259 (CD8, PolyA+)
R+0 (CD8, PolyA+)
R+35 (CD8, PolyA+)
R+104 (CD8, PolyA+)
R+190 (CD8, PolyA+)
R+201 (CD8, PolyA+)
GD−114 (CD8, PolyA+)
GD−66 (CD8, PolyA+)
GD 125 (CD8, PolyA+)
GD 189 (CD8, PolyA+)
GD 204 (CD8, PolyA+)
GD+2 (CD8, PolyA+)
GD+65 (CD8, PolyA+)
GD+137 (CD8, PolyA+)
GD+181 (CD8, PolyA+)
GD+192 (CD8, PolyA+)
L−162 (CD8, Ribodepleted)
L−148 (CD8, Ribodepleted)
L−71 (CD8, Ribodepleted)
FD 76 (CD8, Ribodepleted)
FD 259 (CD8, Ribodepleted)
R+0 (CD8, Ribodepleted)
R+35 (CD8, Ribodepleted)
R+104 (CD8, Ribodepleted)
R+190 (CD8, Ribodepleted)
R+201 (CD8, Ribodepleted)
GD−114 (CD8, Ribodepleted)
GD−104 (CD8, Ribodepleted)
GD−66 (CD8, Ribodepleted)
GD 125 (CD8, Ribodepleted)
GD 189 (CD8, Ribodepleted)
GD 204 (CD8, Ribodepleted)
GD+2 (CD8, Ribodepleted)
GD+65 (CD8, Ribodepleted)
GD+137 (CD8, Ribodepleted)
GD+181 (CD8, Ribodepleted)
GD+192 (CD8, Ribodepleted)
Enrichment score
2
1
0
−1
−2
Subject
Flight
Ground
Control
ReturnType
Ambient
Fresh
Frozen
SampleType
Pre−flight
Flight
Post−flight
Ground
AmbientControl